Swiss RNA Workshop 2020

Session 3, Talk 1

FUS-dependent phase separation initiates double-strand break repair

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Accumulation of DNA damage in neurons is largely described in aging process and neurodegenerative diseases. Recently, RNA-binding proteins (RBPs) have emerged as important effectors of the cellular DNA damage response (DDR). The intrinsically disordered RBP FUS has been shown to play a role in multiple cellular functions, including mRNA transcription, splicing and transport, and in DDR. Besides that, FUS, together with some other RBPs, has been shown to undergo liquid-liquid phase separation (LLPS) under physiological conditions, but its precise molecular functions in DDR and the role of its LLPS remain poorly understood. The aims of this study are to: i) characterise the molecular functions of FUS in the DDR, ii) assess whether a proper repair of double strand breaks (DSB) is dependent on the LLPS of FUS.

Using gene-edited HeLa cell lines, we showed that knock-out of FUS (FUS-KO) sensitised cells to genotoxic stress, impaired DSB repair, and caused changes in DDR pathways. At the molecular level, we found that FUS-KO impaired the recruitment of apical effectors of the DDR to laser-induced DNA damage sites. FUS itself is recruited within seconds to DSBs and is required for the retention of the apical DNA DSBs sensor KU. Mass spectrometry analysis revealed that FUS co-purifies with RBP implicated in DDR, including SFPQ. We demonstrate that LLPS occurs at DNA damage foci and is required for the efficient recruitment of key DDR factors. Finally, LLPS-deficient FUS variants impair the recruitment of KU and SFPQ. Overall, our findings establish for the first time that FUS-dependent LLPS contributes to the activation of the DDR and to the recruitment of DDR proteins at sites of DNA damage.