What is FUS doing to the MDC1-RNF8-RNF168 axis? A possible novel interaction in DNA damage response

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Abstract:
DNA damage occurs continuously inside cells and the integrity of repairing systems is fundamental for genome maintenance. Alterations in these pathways are associated to human diseases such as neurodegenerative disorders and tumours. The most dangerous type of DNA damage are double-strand breaks (DSBs), since they can lead to chromosomal rearrangements. The main actors involved in DSBs repair are known, but their interaction with the multitude of involved proteins is still elusive. The MDC1-RNF8-RNF168 pathway is one of the major axis of the DSBs response, since it mediates the formation of an ubiquitin-based platform that allows the recruitment of DNA damage response (DDR) factors. One of the proteins recruited to DSBs is FUS, a member of the FET family of RNA-binding proteins. FUS variants have been found in amyotrophic lateral sclerosis patients and FUS translocations drive oncogenic transformation in different sarcomas. Upon damage FUS undergoes liquid-liquid phase separation, helping the assembly and coordination of DDR factors. However, its role in the MDC1-RNF8-RNF168 axis has not been explored.

The aim of this project is to investigate the function of FUS in the DSB response, with a focus on its interplay with the MDC1-RNF8-RNF168 axis. In particular, we will take advantage of a FUS-knockout (KO) HeLa cell line and we will investigate FUS role by i) protein-protein interaction studies (co-immunoprecipitation and proximity ligation assay), ii) in vivo recruitment studies, and iii) by assessing DSB response functionality.

Preliminary laser micro-irradiation experiments in Hela cells showed that RNF8 recruitment to DNA damage sites is strongly reduced in the absence of FUS. This effect could be mediated either by a direct interaction between FUS and RNF8 or by an indirect role of FUS in coordinating the formation of the repair complexes. We will investigate both upstream and downstream DDR factors, to identify the specific role of FUS in this axis.

Identification of the role of FUS in the MDC1-RNF8-RNF168 axis will not only shed light on a fundamental cellular mechanism but could help dissect the pathological role of FUS variants in neurodegenerative disorders and in tumours.